Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

Optimal experimental design for discriminating between microbial growth models as function of suboptimal temperature

I. Stamati[a], F. Logist[a], E. Van Derlinden[a], J.-P. Gauchi[b], J. Van Impe[a,*]

[a] *BioTeC & OPTEC, Chemical Engineering Department, KU Leuven, W. de Croylaan 46, 3001 Leuven, Belgium*
[b] *Unit MIA (UR341), INRA, Domaine de Vilvert, 78352 Jouy en Josas, France*

Abstract

In the field of predictive microbiology, mathematical models play an important role for describing microbial growth, survival and inactivation. Often different models are available for describing the microbial dynamics in a similar way. However, the model that describes the system in the *best* way is desired. Optimal experimental design for model discrimination (OED-MD) is an efficient tool for discriminating among rival models.

In this work the T_{12}-criterion proposed by Atkinson and Fedorov (1975) and applied efficiently by Ucinski and Bogacka (2005) and the Schwaab-approach proposed by Schwaab et al. (2008) and Donckels et al. (2009) will be applied for discriminating among rival models for the microbial growth rate as a function of temperature. The two methods will be tested *in silico* and their performances will be compared.

Results from a simulation study indicate that it is possible to validate the case that one of the proposed models is more accurate for describing the temperature effect on the microbial growth rate. Both methods are able to design inputs with a sufficient discrimination potential. However, it has been observed that the Schwaab-approach provides inputs with a higher discrimination potential in combination with more accurate parameter estimates.

Keywords: predictive microbiology, model discrimination, optimal experimental design, dynamic modelling, optimization

*Corresponding author
Email address: `j.VanImpe@cit.kuleuven.be` (J. Van Impe)

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

1. Introduction

The need to find the best model arises when different models are proposed for the same process. For describing the influence of temperature on the microbial growth rate μ_{max} there exist several models in predictive microbiology. Two of these models are the Cardinal Temperature Model with Inflection (CTMI), (Rosso et al., 1993) and the adapted CTMI (aCTMI), (Le Marc et al., 2002), (Bajard et al., 1996)). Whereas the CTMI assumes a one-phase linear relation between $\sqrt{\mu_{max}}$ and the temperature in the suboptimal temperature range, the aCTMI is build from the observation of two phases in this temperature region. As this suboptimal temperature range typically covers the temperature span in which food products are stored, an accurate model description of the growth rate is of highest importance. Up to now, it is assumed that the CTMI is generally valid for all strains. Divergence from this model only has been observed for *Listeria* (Le Marc et al., 2002; Bajard et al., 1996) and *E. coli* K12 (Van Derlinden and Van Impe, 2012).

The main objective of this simulation study is to discriminate CTMI and aCTMI, by performing *in silico* experiments. For performing these *in silico* experiments two specific experimental design procedures for model discrimination will be tested and their performances will be compared. The first one is T-procedure applied effciently by Ucinski and Bogacka (2005), based on the T_{12}-criterion proposed by Atkinson and Fedorov (1975), that leads to non sequential T_{12}-optimal designs. The second one is the Schwaab-procedure based on the approach proposed by Schwaab et al. (2008) and Donckels et al. (2009), that leads to sequential designs. At the T_{12}-criterion the minimum of the sum of squares for the lack of fit of the model is maximized. The Schwaab-approach includes the posterior covariance matrix of the estimated model parameters.

In this simulation study, for the two approaches - the T-procedure and the Schwaab procedure - typical constraints that arise when modelling microbial dynamics are taken into account. These constraints involve, e.g., (i) an a-priori specification of the number of (time-consuming) experiments and (ii) the uncertainty of the actual parameter values as typically only estimates

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

from literature or a preliminary experiment are present. In contrast to a typical experiment design of an arbitrarily chosen set of constant temperature levels, the dynamic experiments designed within this work will be used to efficiently discriminate between these two models.

The paper is structured as follows. In the first part, optimal experimental design for model discrimination is presented covering the two approaches. Afterwards the complementary tasks for the optimal experimental design are sketched out. In the third part, the case study with the two proposed models is outlined followed by the implementation. Finally, in the last part the results from both methods for the discrimination between the two models are presented followed by the conclusions.

2. Optimal experimental design for model discrimination

The procedure for discriminating between two models $\mathcal{M}_1$ and $\mathcal{M}_2$ will be described in this section. The objective function J for model discrimination is typically a discrimination criterion that maximizes a function of the difference between the model predictions. As performing experiments in predictive microbiology is typically time and labour intensive, a practical constraint is to have the same experimental burden, i.e., performing the same number of experiments in both approaches.

2.1. Mathematical model formulation

In a general statistical framework it can be assumed that observations can be repeated for different settings of experimental conditions $T_i(\cdot, \beta_i) \in \mathcal{T} \times B^s, i = 1, \ldots, N_s$ where $\mathcal{T}$ is the set of all measurable functions satisfying $T_{low} \leq T(t) \leq T_{high}$ for all time $t \in [t_0, t_f]$, B is a set of discrete experimental conditions $\beta_i = (\beta_1, \ldots, \beta_s)'$ and N_s is the number of design support points (i.e.,number of designed experimental conditions $T_i(\cdot, \beta_i)$).

The following statistical model is considered at each t_k time instant (Uciski and Bogacka, 2005):

$$y_{ij}(t_k) = \eta(t_k, \theta; T_i(\cdot, \beta_i)) + \varepsilon_{ij}(t_k), \quad i = 1, \ldots, N_s; \quad j = 1, \ldots, r_i. \qquad (1)$$

where $\eta()$ is the true model of the process, sampled at given time instants $t_0 < t_1 < \ldots < t_k < \ldots < t_f$. It is assumed that all the errors $\varepsilon_{ij}(t_k)$ are normal and independent with each other $\forall i, j, k$. Moreover $\forall i, j, k$, $E(\varepsilon_{ij}(t_k)) = 0$

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

and $V(\varepsilon_{ij}(t_k)) = \sigma^2$. $\sum_{i=1}^{N_s} r_i = N$, r_i is the number of repetitions of an experiment located on a support point i, for an experimental condition $(T_i(\cdot), \beta_i)$.

The two competing monoresponse models $\mathcal{M}_1$ and $\mathcal{M}_2$ are expressed by $\eta_1(t, \theta_1; T(\cdot))$ and $\eta_2(t, \theta_2; T(\cdot))$ where $\theta_1 \in \Theta_1 \in \Re^{p_1}$ and $\theta_2 \in \Theta_2 \in \Re^{p_2}$ are vectors of unknown parameters and Θ_1 and Θ_2 are known compact sets. In the current discrimination procedures the initial conditions parameters β_i are excluded, further there will be no repetitions of the experiments, i.e., $r_i = 1 \ \ \forall i$.

2.2. T_{12}-criterion

The first approach (T_{12}-criterion (Ucinski and Bogacka, 2005; Atkinson and Fedorov, 1975)) will be described here. The efficiency of this method is based on the fact that the minimum of the sum of squares for the lack of fit of the model is maximized. Thus, it takes into account the flexibility of the model to fit suitably the responses of the other model. Among others, this criterion has been proven to lead to an increase of the power of the discrimination statistical tests.

In this method the first model $\mathcal{M}_1$ is considered as the true model. Therefore parameter θ_1 is known and can be omitted giving $\eta(\cdot;\cdot) \equiv \eta_1(\cdot, \theta_1; \cdot)$.

The problem of discriminating between the two models is defined by the function (Uciski and Bogacka, 2005)

$$T_{12}(\xi_N) = \min_{\theta_2 \in \Theta_2} \sum_{i=1}^{N_s} w_i \sum_{t_k = t_0}^{t_f} ||\eta(t_k; T_i(\cdot)) - \eta_2(t_k, \theta_2; T_i(\cdot))||^2 \qquad (2)$$

where the design ξ_N is defined by :

$$\xi_N = \left\{ \begin{array}{ccc} (T_1(\cdot)), & \ldots, & (T_{N_s}(\cdot)) \\ w_1, & \ldots, & w_{N_s} \end{array} \right\} \in \Xi. \qquad (3)$$

The experimental conditions $T_i(\cdot)$ represent the design support points, w_i are weights at these support points with $\sum_{i=1}^{n} w_i = 1$ and Ξ is a feasible solution set.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

2.3. Schwaab-approach

The second criterion (Schwaab-approach (Schwaab et al., 2008; Donckels et al., 2009)) that has been used is typically based on a *sequential* approach and will be described below. The primary objective is the increase of the discrimination power but a decrease of the a decrease of the parameter estimate variances is obtained as well, with the use of the posterior covariance matrix of parameter estimates (Schwaab et al., 2008). Differently from the previous method neither of the two models is considered as true. In this approach for sake of clarity ω is used as a reference for an experiment instead of ξ.

For discriminating between model $\mathcal{M}_1$ and $\mathcal{M}_2$, for experiment ω_{Ne+1} defined by $T_{N_e+1}(\cdot)$ and t_k (with N_e the number of available experiments either preliminary or discrimination experiments since it is a sequential approach) the discrimination function, that has to be maximized, is defined at every t_k by:

$$\mathbf{D}_{1,2}(\omega_{N_e+1}) = d_{1,2}^T(\omega_{N_e+1})\mathbf{V}_{1,2}^{-1}(\omega_{N_e+1})d_{1,2}(\omega_{N_e+1}) \tag{4}$$

with:

$$\begin{aligned}
d_{1,2}(\omega_{N_e+1}) &= \hat{\eta_1}(\omega_{N_e+1}, \hat{\theta_1}) - \hat{\eta_2}(\omega_{N_e+1}, \hat{\theta_2}) \\
\mathbf{V}_{1,2}(\omega_{N_e+1}) &= 2\mathbf{V} + \mathbf{V}_1(\omega_{N_e+1}) + \mathbf{V}_2(\omega_{N_e+1}) \\
\mathbf{V}_1(\omega_{N_e+1}) &= \mathbf{B}_1(\omega_{N_e+1})\mathbf{V}_{\theta_1}(\omega_{N_e+1})\mathbf{B}_1^T(\omega_{N_e+1}) \\
\mathbf{V}_{\theta_1}(\omega_{N_e+1}) &= [\mathbf{B}_1^T(\omega_{N_e+1})\mathbf{V}^{-1}\mathbf{B}_1(\omega_{N_e+1}) + \mathbf{V}_{\theta,1}^{-1}(\omega_{N_e})]^{-1}
\end{aligned}$$

In the following formulas t_k is omitted for sake of simplicity. Here, $\hat{\eta_1}(\omega_{N_e+1}, \hat{\theta_1})$ is the *prediction for model* $\mathcal{M}_1$ (similarly for model $\mathcal{M}_2$), $\mathbf{V}_{1,2}(\omega_{N_e+1}) \in \Re^{K\times K}$ is the *posterior covariance matrix* of the differences between model predictions, K is the number of discrete time points t_k, $\mathbf{V} \in \Re^{K\times K}$ is the *covariance matrix of the experimental deviations* and $\mathbf{V}_1(\omega_{N_e+1}) \in \Re^{K\times K}$ is the *covariance matrix of model prediction variations* calculated for model $\mathcal{M}_1$ (and similar for model $\mathcal{M}_2$). The model uncertainty includes the uncertainty on the model predictions and on the measurements (Schwaab et al., 2008; Donckels et al., 2009).

$\mathbf{B}_1(\omega_{N_e+1}) \in \Re^{K\times p_1}$ is the sensitivity matrix that contains the first derivatives of model m responses with respect to its parameters:

$$\left(\frac{\partial\eta_1(\omega_{N_e+1}, \theta_1)}{\partial\theta_1}\right)$$

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

$\mathbf{V}_{\theta_1}(\omega_{N_e+1}) \in \Re^{p_1 \times p_1}$ is the *posterior covariance matrix of model parameter estimates*. It can be seen that $\mathbf{V}_{\theta_1}$ consists two parts, i.e., the covariance matrix of the new designed experiment with experiment condition $T_{N_e+1}(\cdot)$ and the current covariance matrix of the parameter estimates. The covariance matrix of the estimated parameters is approximated by the inverse of the Fisher information matrix (FIM), since the errors are assumed independent (Walter and Pronzato, 1997).

3. Complementary tasks for model discrimination

Apart from the main optimization task there are some complementary tasks for the discrimination. Before the discrimination a preliminary experiment has to be designed for obtaining an initial estimate of the parameters. When an experiment is performed (either preliminary or discriminatory) it provides measurements that can be used in a parameter estimation task. Finally after the design of inputs for the discrimination a model adequacy test can take place for evaluating the two models. These tasks are presented in this section.

3.1. Preliminary experiment

For the T_{12}- criterion it is necessary to have an initial guess for θ_2, which is not needed to be a very good estimate. On the contrary for the Schwaab-approach it is necessary to have not too bad estimates for θ_1 and θ_2. For the comparison of the two methods the initial guess for θ_2 needed for the T-procedure will be the estimated $\hat{\theta}_2$. Furthermore, if in the literature the true values of $\mathcal{M}_1$ are not available, from a practical point of view it is difficult to find the real values, in this scenario the estimated values are used instead. Then, an accurate optimal design is needed.

An initial experiment is required in order to have a good estimate of the unknown parameters. For this the methodology of optimal experimental design for parameter estimation (OED/PE) will be used (Walter and Pronzato, 1997; Van Derlinden et al., 2008; Telen et al., 2012; Pronzato and Walter, 1988). The initial estimates for the OED/PE are based on literature.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

The designed input will provide experimental data with an information content of high quality. The quality of the information can be quantified by the Fisher Information Matrix since the errors will be assumed normal, independent and identically distributed (Walter and Pronzato, 1997).

$$\mathbf{FIM}(\theta) = \sum_{t_i=t_0}^{t_k} \left(\frac{\partial \eta(t_i, \omega_{N_e+1}, \theta)}{\partial \theta}\right)^T \mathbf{Q} \left(\frac{\partial \eta(t_i, \omega_{N_e+1}, \theta)}{\partial \theta}\right) \tag{5}$$

$\mathbf{FIM}(\theta)$ combines information on (i) the error on the measurements ($\mathbf{Q}$ is typically defined as the inverse of the measurement error variance matrix), and (ii) the sensitivities of the model prediction $\eta(t_k, \omega_{N_e+1}, \theta)$ to small variations in the model parameters θ (expressed in the sensitivity matrix $\dfrac{\partial \eta(t_i, \omega_{N_e+1}, \theta)}{\partial \theta}$).

In order to maximize the information in optimal experimental design often a scalar function of the Fisher information matrix is optimized. There are several scalar criteria used in literature. Among others the D-criterion and the E-criterion which aim to maximize the determinant or the minimum eigenvalue of the FIM, respectively (Walter and Pronzato, 1997). In this work the E-criterion ($\max(\lambda_{min}(FIM))$) has been used.

3.2. Parameter estimation

Given an input and the corresponding experimental data, either after the preliminary experiment or a discriminatory experiment, parameters can be estimated. Assuming that the parameters are identifiable (Jacquez and Greif, 1985; Chou and Voit, 2009). Parameters are selected such that the model predictions of $\eta(t_k, \theta; T_i(\cdot))$ fit the observations y_{ij}, at times t_k, as accurately as possible despite the presence of measurement errors. The most common assumption about the probability distribution of the measurement errors is that they are normal,additive, independent and identically distributed. These assumptions typically lead to a *weighted sum of squares* objective ($WSSE$) (Walter and Pronzato, 1997)

$$WSSE(\theta) = \sum_{i=1}^{N_s} \sum_{t_k=t_o}^{t_f} (y_{ij} - \hat{\eta}(t_k, \hat{\theta}; T_i(\cdot)))^T \mathbf{Q}(y_{ij} - \hat{\eta}(t_k, \hat{\theta}; T_i(\cdot))) \tag{6}$$

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

3.3. Model adequacy test

To be able to discriminate between the two models there should be statistical test indicating that one model is better than the other. The use of the χ^2-test can prove a lack of fit (Chen and Asprey, 2003). Since the measurements are assumed to follow a normal distribution with zero mean and known variance σ^2, the $WSSE$ function follows a χ^2 distribution with $N-p$ degrees of freedom. This allows the use of the χ^2 adequacy test (Donckels et al., 2009). If the $WSSE$ value is above the χ^2_{N-p} value (with N the total number of experiment points and p the number of unknown parameters), there is an indication of *lack of fit*.

A selection method is to use a selection criterion such as the corrected Akaike information criterion (AIC_c). The general Akaike information criterion is defined as:

$$AIC = -2ln(L(\theta)) + 2p \tag{7}$$

where $L(\theta)$ is the likelihood of the sample. The parameters are estimated through least squares and there is the assumption of normal distribution with zero mean and known variance σ^2. The mean square error is defined by:

$$\text{MSE} = \frac{\sum_{i=1}^{N} e_i^2}{N-p} \tag{8}$$

with e_i being the residuals.

If we assume normal errors with constant variance the AIC can be expressed as:

$$AIC = Nlog(\text{MSE}) + 2p \tag{9}$$

When N the number of measurements is small, the AIC_c is more statistically rigorous to use and is defined as (Burnham and Anderson, 2002; Hurvich and Tsai, 1989):

$$AIC_c = AIC + \frac{2p(p+1)}{N-p-1} \tag{10}$$

By using Equation 9 the AIC_c becomes:

$$AIC_c = Nlog(\text{MSE}) + 2p + \frac{2p(p+1)}{N-p-1} \tag{11}$$

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

The model with the lower AIC_c value can be selected and fits the data more accurately.

An important remark is that the criteria above are in theory not valid in the presence of order constraints on the parameters. In such a situation adaptations as described in Silvapulle and Sen (2005); Kuiper et al. (2011) have to be considered. However, in practice when only simple constraints are present that hardly have an influence, hardly any difference between the criteria will be noticeable and the approximation will be small. Hence, also the conclusion on the model selection will not be affected.

4. Case study

In predictive microbiology a two step modelling approach is classically used. The first step consists of a *primary model*. This model describes the microbial growth, survival or inactivation under constant environmental conditions. Whereas in the second step, the parameters of the primary model are described by a *secondary model* as a function of changing environmental conditions, e.g., temperature, pH and water activity (Baranyi and Roberts, 2004). When combining both primary and secondary model the microbial behaviour can be described in a dynamic environment. There exist several primary models in literature, in this work the growth model of Baranyi and Roberts (1994), describing the cell density as a function of time, is used:

$$\begin{aligned} \frac{dn(t)}{dt} &= \frac{Q(t)}{Q(t)+1} \cdot \mu_{max}(T(t)) \cdot [1 - \exp(n(t) - n_{max})] \\ \frac{dQ(t)}{dt} &= \mu_{max}(T(t)) \cdot Q(t) \end{aligned} \tag{12}$$

with $n(t)$ [ln(CFU/mL)] the cell density at time t [h], n_{max} [ln(CFU/mL)] the maximum value for $n(t)$ and μ_{max} [1/h] the maximum specific growth rate. $Q(t)$ is a measure for a physiological state of the cells. For this work $Q(t)$ is excluded (see Van Derlinden et al. (2010) for details) and thus the model is reduced to:

$$\frac{dn(t)}{dt} = \mu_{max}(T(t)) \cdot [1 - \exp(n(t) - n_{max})] \tag{13}$$

The microbial growth rate as a function of temperature (secondary model) can be described by the CTMI and the aCTMI. For simplicity the tempera-

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

ture evolution $T(t)$ will be noted as T in the following.
The CTMI is described by:

$$\mu_{max}(T) = \gamma(T) \cdot \mu_{opt} \tag{14}$$

with

$$\gamma(T) = \begin{cases} 0 & T \leq T_{min} \text{ or } T \geq T_{max} \\ \dfrac{(T - T_{min})^2 (T - T_{max})}{(T_{opt} - T_{min})(\gamma_A(T) - \gamma_B(T))} & T_{min} < T < T_{max} \end{cases}$$
$$\gamma_A(T) = (T_{opt} - T_{min})(T - T_{opt})$$
$$\gamma_B(T) = (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T) \tag{15}$$

The parameters included in this model are the three cardinal temperatures $T_{min}[^o\text{C}]$, $T_{opt}[^o\text{C}]$ and $T_{max}[^o\text{C}]$ (i.e., the minimum, optimum and maximum temperature for growth, respectively) and $\mu_{opt}[1/h]$ (the maximum specific growth rate at T_{opt}).

The aCTMI is described in a similar way as the CTMI but with a different $\gamma(T)$ function:

$$\gamma(T) = \begin{cases} 0 & T \leq T_{min} \text{ or } T \geq T_{max} \\ \dfrac{(T_c - T_1)^2 (T_c - T_{max})}{(T_{opt} - T_1)(\gamma_C(T) - \gamma_D(T))} \left(\dfrac{T - T_{min}}{T_c - T_{min}}\right)^2 & T_{min} < T \leq Tc \\ \dfrac{(T - T_1)^2 (T - T_{max})}{(T_{opt} - T_1)(\gamma_E(T) - \gamma_F(T))} & T_c < T < T_{max} \end{cases}$$

$$\gamma_C(T) = (T_{opt} - T_1)(T_c - T_{opt})$$
$$\gamma_D(T) = (T_{opt} - T_{max})(T_{opt} + T_1 - 2T_c)$$

$$\gamma_E(T) = (T_{opt} - T_1)(T - T_{opt})$$
$$\gamma_F(T) = (T_{opt} - T_{max})(T_{opt} + T_1 - 2T) \tag{16}$$

Apart from the previous four parameters the adapted model is defined also by $T_c[^o\text{C}]$ the so-called change temperature and $T_1[^o\text{C}]$ the intersection point between the first linear part and the temperature axis. In Figure 1 the $\sqrt{\mu_{max}}$ versus the temperature is displayed for the two models, and their

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

difference in the region of T_{min} can be seen.

Although the notation used above may be confusing, the choice has been made to keep all parameter names as close as possible to the names in the original papers. Hence, the temperature at which maximum growth occurs (T_{opt}), and the maximum allowable temperature at which growth ceases (T_{max}) have an identical meaning in both models. The minimum temperature required for growth in the CTMI model is $T_{min,CTMI}$. This temperature has been renamed to T_1 in the aCTMI. However, the lowest temperature at which growth is possible in the aCTMI, is called $T_{min,aCTMI}$ and the temperature where the deviation starts between the CTMI and aCTMI model is T_c.

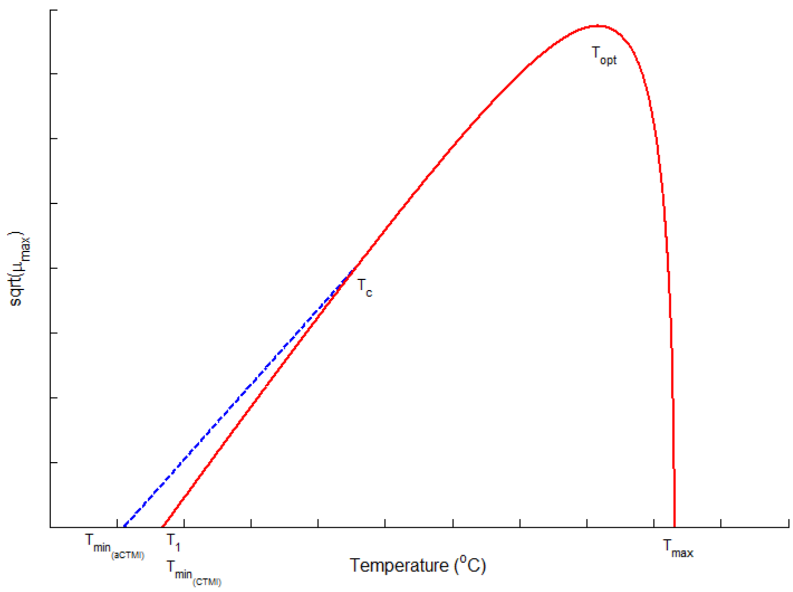

Figure 1: The maximum specific growth rate $\sqrt{\mu_{max}}$ as a function of temperature, as described by the CTMI (-) and aCTMI (- -) models.

5. Implementation

The aCTMI coincides with the CTMI if parameters $T_c[^oC]$ and $T_1[^oC]$ are well chosen (see Figure 1), i.e., if $T_c = T_1 = T_{min_{CTMI}}$ than T_c and T_1 also

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

equal $T_{min_{aCTMI}}$. Hence, having a T_c lower than T_1 or $T_{min_{aCTMI}}$ does not make sense. So, the following inequalities are present:

$$T_c > T_1 > T_{min,aCTMI} \tag{17}$$

In contrast, the CTMI does not always coincide with the aCTMI as the aCTMI is also able to describe the deviating $\mu_{max}(T)$-relation in the suboptimal temperature region. This feature will be verified with the use of model discrimination techniques. Assuming aCTMI is the *correct* model, *in silico* data have been created and used as measurement data.

When applying OED-MD for this case study, it is important to notice that discrimination between the two models is not possible when taking the opposite approach, i.e., assuming that the CTMI is the correct model. Since the CTMI is a subclass of the aCTMI, the additional parameters of the aCTMI (T_c and T_1) can be chosen such that the aCTMI coincides with the CTMI. Thus separation of these models is never possible in this opposite approach. In other words the resulting AIC_c values will be very close indicating that the models are not to be discriminated.

Figure 4 sketches the general frame of the study. The two approaches will be evaluated for an identical number of experiments, i.e., two. The T-criterion simultaneously designs two experiments while the Schwaab method designs them sequentially. In the latter method, an initial experiment is first designed and performed and afterwards a second one is proposed and performed. In addition as the T-criterion assumes the true parameters of the aCTMI are known, two different scenarios are tested. In the first scenario (Scenario 1) the true parameters of the aCTMI are known, while in the second scenario (Scenario 2) the true parameters are unknown and only estimates based on a preliminary experiment are available. Consequently, this frame serves the general aim of the story. Both approaches will be tested while evaluating the effect of practical limitations. This includes a fixed number of (time consuming) experiments and unavailability of the true parameter values in practice. To corroborate the results, simulations have been repeated 100 times.

5.1. Input profile

The input profile (experimental condition $T_i(\cdot)$) is parametrized with four degrees of freedom (Figure 2): T_1 [^{o}C] the initial temperature, t_s [h] the

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

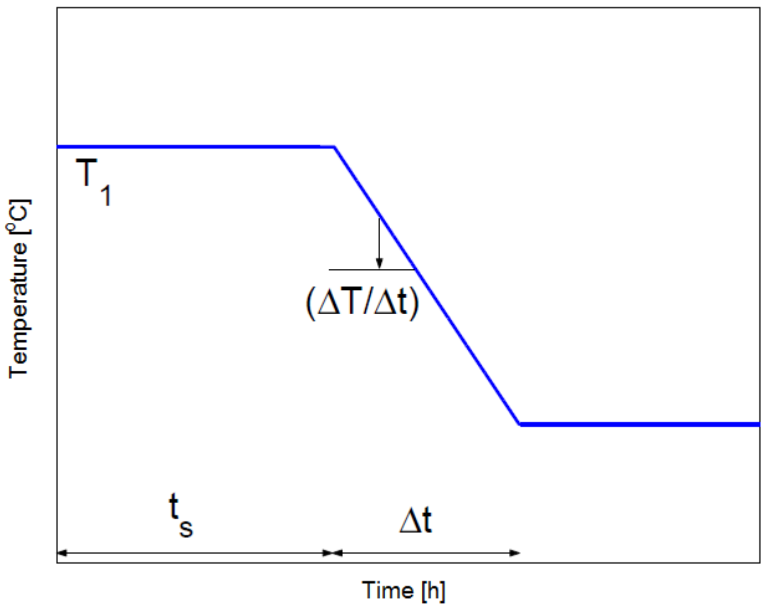

Figure 2: Parameterized temperature profile characterized by four input parameters: T_1 [^{o}C] the initial temperature, t_s [h] the time at which the increase or decrease in temperature starts, $\Delta T/\Delta t$ [^{o}C/h] the rate of temperature change and Δt [h] the duration of the temperature change (Van Derlinden et al., 2010).

time at which the increase or decrease in temperature starts, $\Delta T/\Delta t$ [^{o}C/h] the rate of temperature change and Δt [h] the duration of the temperature change (Van Derlinden et al., 2010). This input profile is optimized in the two discrimination approaches. The temperature is allowed to be in the range of $[0, 45]^o$C, the total time is 38 hours with a sampling time of 1 hour.

5.2. Measurement data and parameters

The parameters used for generating the *in silico* measurements are $\mu_{opt} = 2.41$ 1/h, $T_{min} = 5.67^o$C, $T_c = 23^o$C and $T_1 = 12.3^o$C (Van Derlinden and Van Impe, 2012). The error added to the predicted data (for generating the *in silico* measurements) has a variance $\sigma^2 = 3.27 \cdot 10^{-2}$ (see Van Derlinden et al. (2008) and the references therein). It has to be noted that in general obtained results may depend on the numerical values used. However, given the underlying microbial process, most parameters have a microbial

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

interpretation and can be found in a (rather) limited range. As a result, typical values for the parameters and the noise have been selected, which are based on more than 15 years of experimental experience within the team. The two models differ in the temperature region below T_{opt}, therefore for this work the temperature parameters T_{opt} and T_{max} are identical for both models and based on previous estimations, i.e., $T_{opt} = 40.85^o$C and $T_{max} = 46.54^o$C (Van Derlinden et al., 2008). For the CTMI, two parameters are unknown (i.e., μ_{opt} and T_{min}) and thus $p_2 = 2$, whereas four parameters ($p_1 = 4$) are assumed to be unknown for the aCTMI (i.e., μ_{opt}, T_{min}, T_c and T_1). Although the current study focusses on an in silico investigation before doing real experiments which are typically time consuming and labour intensive, one experimental dataset is added in Figure 3 as an illustration.

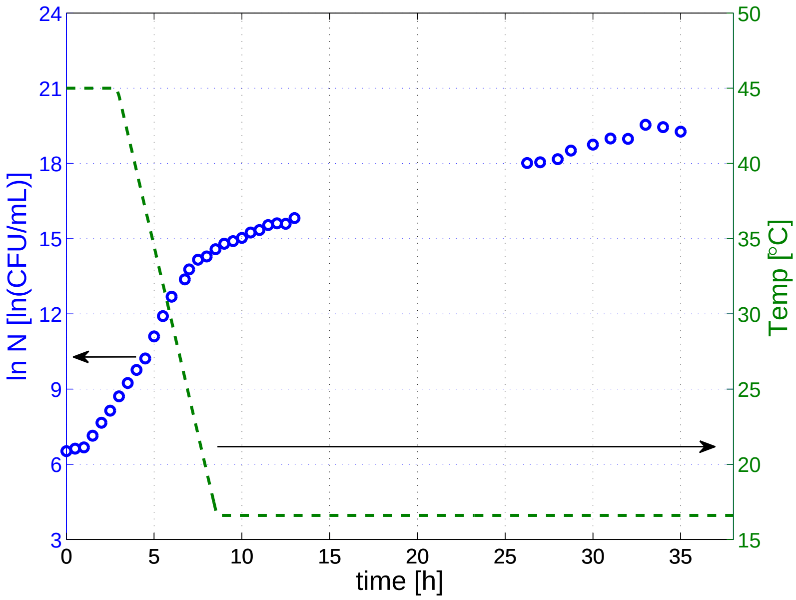

Figure 3: Experimental dataset for *E. coli* K12 based on Van Derlinden et al. (2008): temperature profile and evolution of (the logarithm of) the cell density.

5.3. T-procedure

In this subsection the steps for the T-procedure, based on the T_{12}-criterion, will be presented (sketched in Figure 4). In the T-procedure the designed

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

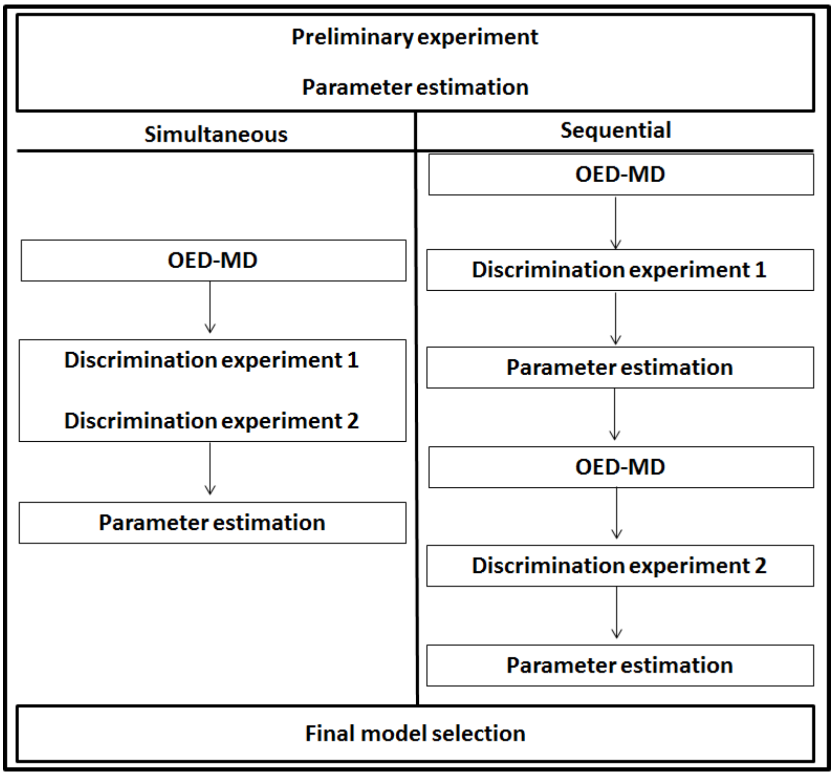

Figure 4: Steps required for discriminating between two models using the two methods T-procedure (simultaneous) and the Schwaab-procedure (sequential).

experimental inputs are obtained through a direct approach.

The first step is to design the preliminary experiment. As described in Section 3.1 optimal experimental design for parameter estimation will be used. By performing this experiment experimental data are available. Afterwards the parameters can be estimated as described in Section 3.2.

The next step is to design the discrimination experiments (Section 2.2). The function (2) should be maximized for obtaining the designed support points and weights (see Equation (3)). To simplify the maximization, the problem is relaxed as explained in (Ucinski and Bogacka, 2005).

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Let

$$M(\xi_N^{[l]}, \theta_2) = \sum_{i=1}^{N_s} w_i \sum_{t_k=t_0}^{t_f} ||\eta(t_k; T_i(\cdot)) - \eta_2(t_k, \theta_2; T_i(\cdot))||^2 \tag{18}$$

1. Choose number of support points N_s that must be ≥ 2. For a more rigorous approach it should be necessary to test $N_s = 2, 3, 4 \cdots$. For instance, see Example 2 in Atkinson & Fedorov (Atkinson and Fedorov, 1975) where $N_s = 4$. In this work $N_s = 2$ is used and it can be seen in the results that it is enough for having a clear conclusion.
2. Choose an initial value for parameter θ_2 and define the first solution set $(l = 1)$ for θ_2 parameter, $\mathbb{Z}^{[1]} = \left\{\theta_2^{[1]}\right\}$.
3. Choose the first $\xi_N^{[0]}$, choose uniform $w_i = 1/N_s, \quad \forall i$.
4. At step l solve:

$$\xi_N^{[l]} = Arg\left\{\max_{\xi\in\Xi}\left[\min_{\theta_2\in\mathbb{Z}^{[l]}} M(\xi_N^{[l]}, \theta_2)\right]\right\} \tag{19}$$

5. Solve the minimization problem

$$\theta_2^{[l+1]} = Arg\left\{\min_{\theta_2\in\Theta_2} M(\xi_N^{[l]}, \theta_2)\right\} \tag{20}$$

6. If $M(\xi^{[l]}, \theta_2^{[l+1]}) \leq (1-\varepsilon)\min_{\theta_2\in\mathbb{Z}^{[l]}}\left[M(\xi^{[l]}, \theta_2)\right]$, where ε is a small predetermined constant, then $\xi^{[l]}$ is a maxmin solution; else include $\theta_2^{[l+1]}$ in $\mathbb{Z}^{[l]}$, increase l and go to step 4.

After obtaining the optimal input profiles the experiments can be performed and the parameters can be estimated.

Finally, a model adequacy test can be performed and the corrected AIC_c criterion value is calculated.

5.4. Schwaab-procedure

For discriminating between two models using the Schwaab-approach the following steps should be followed (sketched in Figure 4). This procedure is a sequential for the optimal design for model discrimination. For a consistent comparison there will be also two discrimination experiments since $N_s = 2$

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

in the T-procedure. The preliminary step is performed as in the T-procedure.

The next step is to design the first discrimination experiment (Section 2.3) and perform it. Using the obtained observations together with the observations of the preliminary experiment the parameters are re-estimated.

A second discrimination experiment is designed as previously and the parameters are re-estimated using the observations of the three different experiments (preliminary and two discrimination).

Finally a model adequacy test is performed and the AIC_c criterion value is calculated as in T-procedure.

5.5. Computer tools

The parameter estimation is performed with the `lsqnonlin` matlab function from the optimization toolbox. This function solves a least squares problem using the trust-region-reflective algorithm (Coleman and Li, 1996).

For the optimal experimental design the maximization problem is solved with the `patternsearch` matlab function from the global optimization toolbox in combination using a multi-start approach. This function finds the minimum of the objective function using a pattern search algorithm (Audet and Dennis Jr, 2003).

For the minimization problem within the T-procedure (for estimating the parameters of the second model) also the `lsqnonlin` function is used in a multi-start approach.

From computational point of view the T-procedure is more complicated since it involves a max min problem and thus takes a longer computing time. Whereas the Schwaab-procedure although containing the sensitivity functions is of shorter time. This is also explained through the fact that in the T-procedure 10 design parameters are to be optimized (for every support point (total 2) the input profile parameters T_1, t_s, $\Delta T/\Delta t$ and Δt as well as the weight w_i) whereas in the Schwaab-procedure only 4 at a time (the four input profile parameters).

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

T_1 [^{o}C] the initial temperature, t_s [h] the time at which the increase or decrease in temperature starts, $\Delta T/\Delta t$ [^{o}C/h] the rate of temperature change and Δt.

6. Results

The T_{12} design criterion assumes that the parameters of aCTMI are known exactly. This is a theoretical assumption and does not always reflect reality. Therefore two scenarios have been taken into consideration. In the first scenario the parameters used for the aCTMI are the real ones, thus the true θ_1 is used, referred to as $\hat{\theta}_1^*$, this is possible since the experiment is *in silico*. Whereas in the second scenario the parameters used for the aCTMI are the estimated parameters obtained after the preliminary experiment, thus a $\hat{\theta}_1$ is used. Through this approach both cases, theoretical and practical, are taken in consideration for the comparison. Also it will reveal the robustness of the T_{12}-criterion and its sensitivity with respect to not knowing the optimal parameters.

After the preliminary experiment both methods will provide designed inputs for discrimination.For both methods two additional experiments will be designed. By the Schwaab-procedure after the design of the first discriminatory input the experiment is performed *in silico* and the parameters for the two models are re-estimated before the design of the second discrimination experiment.

It has to be noted that the AIC_c criterion is not valid in the presence of order constraints on the parameters and extensions should be considered Silvapulle and Sen (2005); Kuiper et al. (2011). Nevertheless, the AIC_c criterion will still be used here (in an approximative way) as the error made can be assumed small since the constraints (17) hardly have any effect (as will be shown later). Hence, this approximation can be assumed not to affect the final decision on the model discrimination.

6.1. Preliminary experiment

As for both scenarios the same preliminary experiment will be used, the E-criterion is applied for the parameters of aCTMI. This is to ensure that in the second scenario the estimated parameters of aCTMI obtained after

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

performing only one experiment are as close to the original values as possible. This is important because they will be used in the discrimination procedure. The obtained parameters from this experiment can be seen in Table 1.

Table 1: Estimated parameter values after preliminary experiment

model	μ_{opt}[1/h]	T_{min}[^{o}C]	T_c[^{o}C]	T_1[^{o}C]
original aCTMI	2.41	5.67	23.00	12.30
	preliminary experiment			
CTMI	2.03	8.59		
aCTMI	2.63	5.76	24.63	14.04

6.2. First scenario

In these series of experiments the parameter of the aCTMI are exactly known (since the experiments are *in silico*).

6.2.1. Discrimination using the T-procedure

The real parameters are used for aCTMI, and as an initial guess for the discrimination procedure the estimated parameters are used for CTMI. The optimization is performed as described in Section 5.3 and the resulting support points (temperature profiles) are displayed in Figure 5 together with the preliminary input.

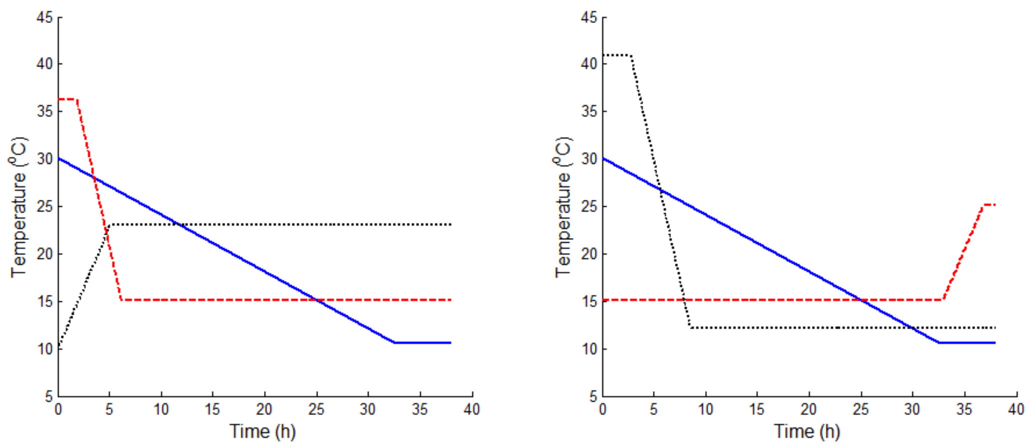

Figure 5: Optimal temperature profiles obtained for the first scenario through T-procedure (left) and Schwaab-procedure (right). Preliminary experiment (-), first discrimination (- -) and second discrimination (.).

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

The two new designed inputs are preformed *in silico*. The observations obtained from all three input profiles are used to re-estimate the parameters of the CTMI. The obtained parameter estimates for the CTMI are $\hat{\mu}_{opt} = 2.15$ [1/h] and $\hat{T}_{min} = 9.20$ [°C]. The parameters of aCTMI are not estimated and the original values are used instead, as these series are under the assumption that the parameters of aCTMI are known.

The model predictions for the two models CTMI and aCTMI together with the corresponding pseudo-measurements from the three experiments are displayed in Figure 6. It can be seen that the model predictions in Figure 6 differ. The aCTMI can describe the measurement data more accurately and CTMI can be discriminated. This can be further confirmed by the test results in Table 2. The $WSSE$ value of aCTMI is below the χ^2-value and thus the fit is accepted in contrast for CTMI where there is an indication of lack of fit. Moreover, the AIC_c value of the CTMI is significantly larger than the AIC_c value of aCTMI and thus it can be selected.

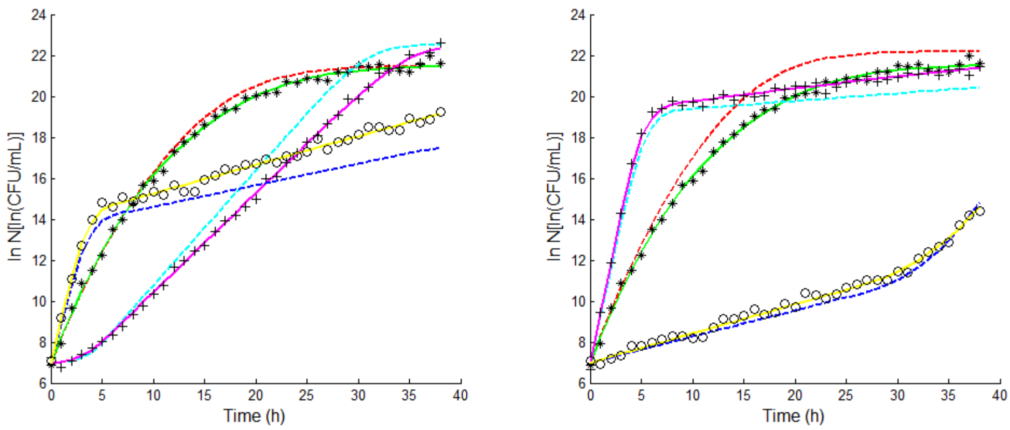

Figure 6: (First scenario) CTMI (- -) and aCTMI (-) model predictions together with pseudo-measurements (preliminary experiment (*), first discrimination experiment (+) and second discrimination experiment (o)) for the T-procedure (left) and Schwaab-procedure (right).

6.2.2. Discrimination using the Schwaab-procedure

As in the above subsection the parameters used for the aCTMI are the real values whereas the parameters for the CTMI are the estimated values.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Table 2: Model adequacy test results and AIC_c criterion values using the T-procedure and Schwaab-procedure.

a. First scenario

model	$WSSE$	χ^2_{N-p}	AIC_c
T-procedure			
CTMI	2550	141	-34
aCTMI	121	139	-383
Schwaab-procedure			
CTMI	1854	141	-71
aCTMI	115	139	-388

b. Second scenario

model	$WSSE$	χ^2_{N-p}	AIC_c
T-procedure			
CTMI	2217	141	-50
aCTMI	472	139	-224
Schwaab-procedure			
CTMI	1908	141	-67
aCTMI	126	139	-378

The corresponding variance matrices are calculated accordingly.

A first discrimination experiment is designed as presented in Section 2.2. The resulting temperature profile (Figure 5) has been applied *in silico*. The parameters of CTMI are re-estimated using the observations of both the preliminary and the first designed experiment resulting $\hat{\mu}_{opt} = 2.58$ [1/h] and $\hat{T}_{min} = 12.18$ [^{o}C]. The parameters of aCTMI are not estimated and instead the real values are adopted.

The parameter values are updated and an additional discriminatory experiment will be designed. The new obtained temperature profile can be found in Figure 5. A new experiment is performed *in silico* with the resulting temperature profile. Using the previous observations together with the new obtained, the parameters of CTMI are re-estimated. The obtained parameter estimates are $\hat{\mu}_{opt} = 2.58$ [1/h] and $\hat{T}_{min} = 12.22$ [^{o}C]. The model predictions and the corresponding observations can be seen in Figure 6. The results from the adequacy test can be found in Table 2.

As in the results with the T-procedure the discrimination is possible with similar discrimination values as observed from Table 2. From this it can be concluded that in the first scenario the two methods perform almost identically, and the discrimination can be achieved with both methods.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

6.3. Second scenario

As mentioned above in these series of experiments the parameters of both models are estimated after the preliminary experiment. The estimated parameters can be found in Table 3. There is a difference from the original values although the experiment was designed for estimating the parameters.

Table 3: Estimated parameter values in the second scenario

model	μ_{opt}[1/h]	T_{min}[^{o}C]	T_c[^{o}C]	T_1[^{o}C]
original aCTMI	2.41	5.67	23.00	12.30
	preliminary experiment			
CTMI	2.03	8.59		
aCTMI	2.63	5.76	24.63	14.04

T-procedure

	after two discrimination experiments			
CTMI	1.91	7.27		
aCTMI	2.41	6.29	24.35	12.49

Schwaab-procedure

	after one discrimination experiment			
CTMI	2.61	12.34		
aCTMI	2.42	5.81	23.59	12.48
	after two discrimination experiments			
CTMI	2.27	8.55		
aCTMI	2.42	5.62	22.97	12.37

6.3.1. Discrimination using T-procedure

Having the parameter estimates the discrimination procedure can start. The obtained estimated parameter values are used for aCTMI (thus $\hat{\theta}_1$) whereas for CTMI they are used as initial values for the optimization ($\hat{\theta}_2^0$). Following the procedure explained in Section 5.3 two support points are obtained. The resulting support points will be the two temperature profiles used for the discrimination as seen in Figure 7. The temperature profiles in this figure are different from Figure 5 and this because different parameter values for aCTMI have been used in the optimization. For the first scenario the parameters for aCTMI are the original ones and can be found in Table 3

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

first row, whereas for the second scenario the parameters for aCTMI are the estimated ones, which can be found in Table 3 in the third row. Between the two figures it can also be observed that the Schwaab approach gives similar results for both scenarios confirming that it can overcome the presence of uncertain parameters.

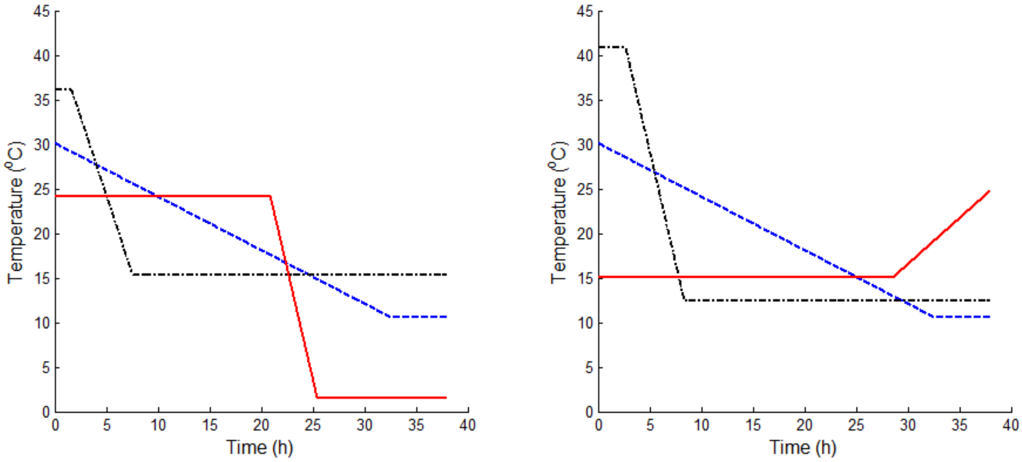

Figure 7: Optimal temperature profiles obtained for the second scenario through T-procedure (left) and Schwaab-procedure (right). Preliminary experiment (-), first discrimination experiment (- -) and second discrimination experiment (.).

These two experiments will be performed *in silico*. The parameters of both models are re-estimated based on the three available experiments (i.e., the preliminary and two discrimination experiments), and can be seen in Table 3.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

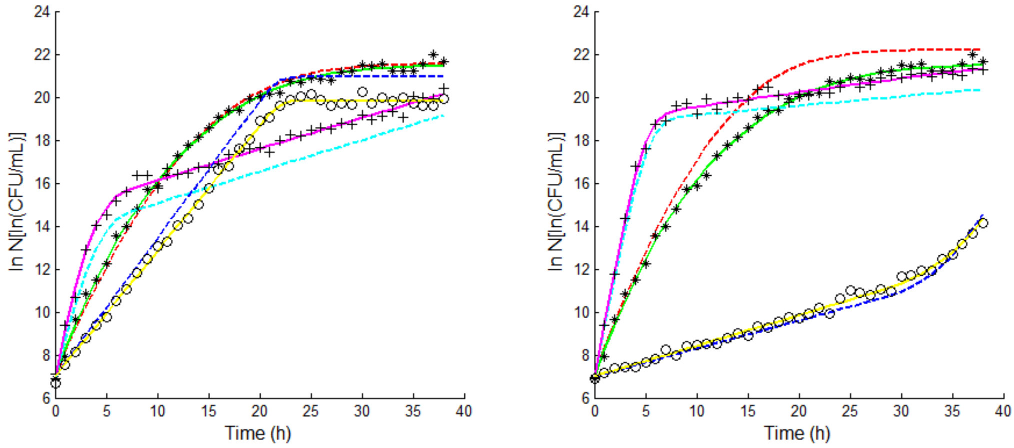

Figure 8: (Second scenario) CTMI (- -) and aCTMI (-) model predictions together with pseudo-measurements (preliminary experiment (*), first discrimination experiment (+) and second discrimination experiment (o)) for the T-procedure (left) and Schwaab-procedure (right).

It can be seen that the model predictions in Figure 8 differ. By studying the test results in Table 2 it can be observed that indeed the two models differ and can be discriminated since the AIC_c value of the CTMI is much higher than the value of the aCTMI. The higher value of the $WSSE$ of aCTMI is expected since the parameters of the aCTMI are estimated. The parameter values in Table 3 are as well an indication that additional experiments are required for identifying the parameters more accurately.

6.3.2. Discrimination using Schwaab-procedure

The parameter values obtained from the preliminary experiment will be used as initial parameter values together with the corresponding variance-covariance matrices. A first discrimination experiment will be designed and the new temperature profile will be used for performing an experiment *in silico*. The two measurement sets (from the preliminary and first discrimination experiment) will be used for re-estimating the parameters. The resulting parameters can be seen in Table 3.

A new discrimination experiment will be designed as previously using the updated parameter values. The resulting temperature profile together with

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

the two previous profiles can be found in Figure 7.

The designed input is applied and the resulting measurement points will be used together with the previous measurements to estimate the parameters. The resulting parameters can be found in Table 3. At this point a model adequacy test can be performed as to evaluate the discrimination.

As observed in Figure 8 the two model predictions differ. The aCTMI is describing the measurement data more accurately, whereas the CTMI fails to fit the data. This can be confirmed by the data in Table 2, where the aCTMI shows acceptable $WSSE$ value in contrast with the CTMI. Moreover the AIC_c value of the CTMI is significantly larger than the value of the aCTMI and thus the CTMI can be excluded. The parameter values as presented in Table 3 show that the parameters of the aCTMI are converging to the original values throughout the experiments. Thus this confirms that the Schwaab-procedure can provide informative inputs both for discriminating between the models and achieving better estimates.

6.4. Additional results

For strengthening the above results additional simulations have been done. For both scenarios the preliminary experiment is taken granted.

For the T-procedure 100 discrimination experiments (see Figure 4 left, double block Discrimination experiment) have been performed *in silico*. These experiments resulted 100 different parameter estimations for the final fit and evaluation of the discrimination. Note that for each of these 100 cases, a single final parameter estimation is performed based on the data of the (sole) preliminary experiment and the data resulting from both experiments for discrimination.

For the Schwaab procedure in order to have 100 final estimations ten discrimination experiments (first block Discrimination experiment 1 on the right) have been performed *in silico* resulting in ten different parameter estimations. For each of this ten parameter sets a second discrimination experiment has been designed (second block OED-MD on the right). Every of these ten discrimination experiments has been performed *in silico* ten times. This results in 100 different final fits (last parameter estimation block on the

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

right). So, every parameter estimation involves the sole preliminary experiment, as well as two consecutive discrimination experiments.

Through this approach the final model selection can be done among 100 different experimental sets.

For the first scenario in which the parameters of aCTMI are known for both methods the AIC_c values for the two models from the full set of experiments are compared. For an easier comparison of the two values $AIC_{c_{CTMI}}$ and $AIC_{c_{aCTMI}}$ the ratio is calculated and illustrated in a histogram that can be seen in Figure 9. Also a Gaussian curve has been fitted for illustrative reasons. It can be seen that both methods achieve discrimination in all cases as a ratio larger than 2 can be taken as a threshold. The T-procedure gives ratios in the span of 7 to 14 with an average of 10.5, while the corresponding values for the Schwaab procedure are in the range of 5 to 6 with an approximate average of 5.5. Hence, the T-procedure can be considered to achieve a better discrimination.

For the second scenario where the parameters of aCTMI were estimated after a single experiment instead of assumed to be known, the AIC_c ratio is shown in Figure 10. On average the ratios are lower indicating a lower discriminatory power. For the T-procedure, the range is now between 6 and 10, with an approximate average of 8. The values for the Schwaab approach can be found in the interval between 4.2 and 5.2, with an average of 4.7. However as these values still largely exceed the threshold value of 2, discrimination can be assumed.

Additionally the estimated parameters of aCTMI after the final fit are displayed in a histogram in Figures 11 and 12 for the T-procedure and the Schwaab procedure, respectively. For the T-procedure the real parameter value is not included in the range for two out of the four estimated parameters. In contrast to this in the Schwaab approach the real parameter values are included in all four ranges. These figures corroborate the previous results that in the second scenario both methods can provide inputs for discrimination but that the Schwaab procedure obtains parameter estimates closer to the original values. It has to be noted that an increase in parameter accuracy can be obtained by performing an additional experiment designed for parameter estimation.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

From the results above, it can be observed that the difference between the estimated nominal values for the three temperatures (i.e., around 6^{o}C, 12^{o}C and 24^{o}C for $T_{min,aCTMI}$, T_1, and T_c, respectively) is many times larger than the standard deviations on the estimates, which are typically less than 1^{o}C. Consequently, in practice the simple inequality constraints (17) have hardly any influence and can easily be omitted. So, the approximation by AIC_c can be assumed accurate and, hence, the final decision which model to select, will not be affected.

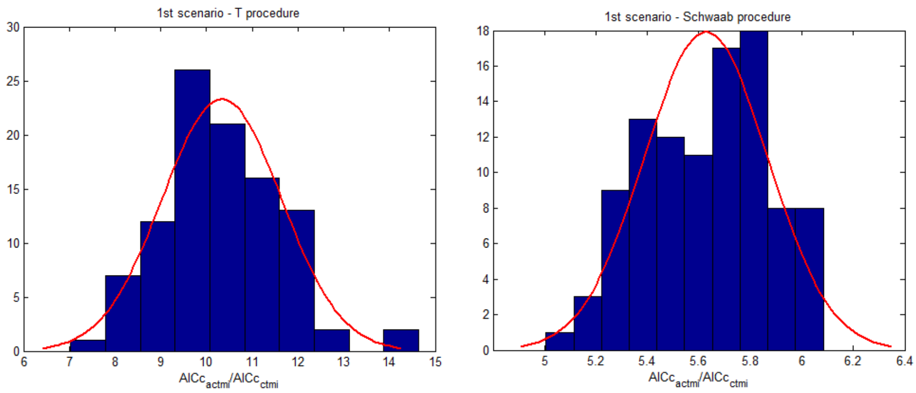

Figure 9: AIC_c ratio for the first scenario after 100 experiments obtained with the T-procedure (left) and Schwaab procedure (right).

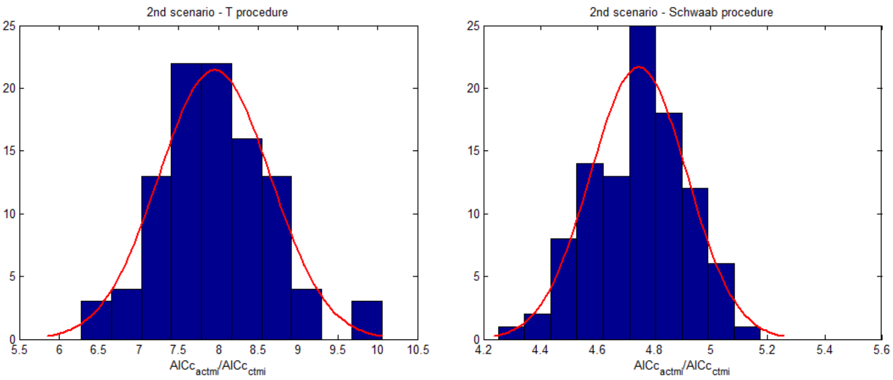

Figure 10: AIC_c ratio for the second scenario after 100 experiments obtained with the T-procedure (left) and Schwaab procedure (right).

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

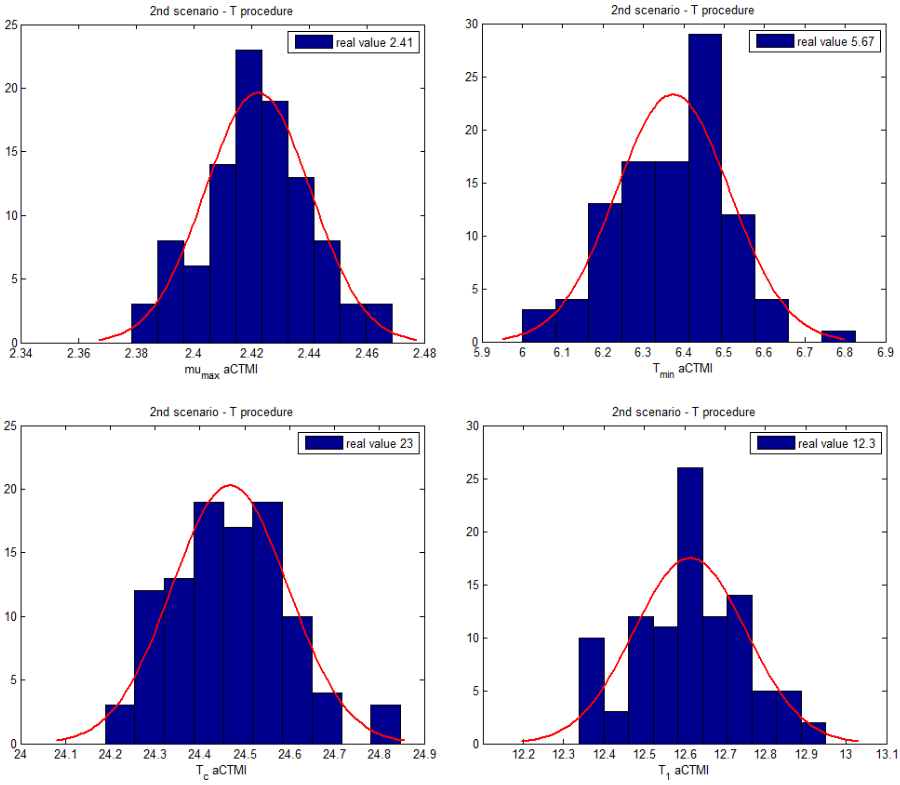

Figure 11: aCTMI parameter values for the second scenario obtained after 100 experiments with the T-procedure.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

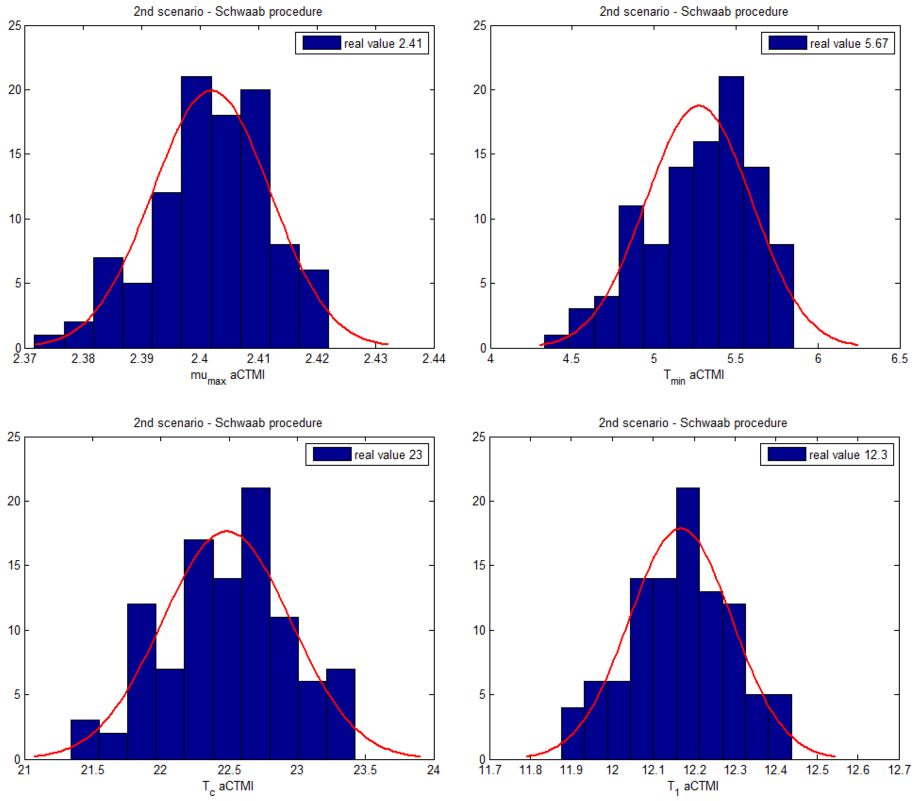

Figure 12: aCTMI parameter values for the second scenario obtained after 100 experiments with the Schwaab procedure.

6.5. Guidelines

Since the aim of the paper is to evaluate in a simulation study two model discrimination approaches taking into account typical constraints, some practical guidelines are to be discussed. When performing experiments in the lab the real model parameters are not known exactly, more often only estimates from literature or previous experiments are available. Therefore the second scenario is more realistic. Furthermore, considering that performing experiments is expensive, the lowest number of experiments possible is preferred. Summing up these facts and having an overview of the results the Schwaab method is recommended for performing model discrimination in the lab. This is because the Schwaab method can provide discriminative inputs and overcome the uncertainty of the parameters using less experiments. Also computationally the Schwaab procedure is less demanding.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

Postprint version of paper published in Mathematical Biosciences 2014, vol. 250, pages 69-80.
The content is identical to the published paper, but without the final typesetting by the publisher.

7. Conclusions

Between the two models CTMI and aCTMI, the question of the *best* model arises. In this work, Optimal Experimental Design for Model Discrimination (OED-MD) is applied and its performance to discriminate between the two models is studied and evaluated on a simulation level. The results from the simulation study show that, if the aCTMI can describe more accurately the region around T_{min}, it is possible to extract this information using OED-MD. In addition, two criteria for model discrimination are compared, i.e., the T_{12}-criterion proposed by Atkinson and Fedorov Atkinson and Fedorov (1975) and applied efficently by Ucinski and Bogacka Ucinski and Bogacka (2005) the Schwaab-approach used by Schwaab et al. Schwaab et al. (2008) and Donckels et al. Donckels et al. (2009).

The results shown in this paper indicate that both methods can provide inputs for discriminating between the two models aCTMI and CTMI. Although in theory it can be assumed that the real parameters are known, this can not be in practice. In reality the parameters of a model are not exactly known but can be estimated. Preliminary experiments can be designed to give accurate parameter estimates, although the parameters will always be estimated with a small uncertainty. Both methods perform almost identically to select a good model between the aCTMI and CTMI. However, the Schwaab procedure was found to converge closer to the original parameter values and requires less computational effort. Obviously the T-procedure was not defined for obtaining good estimates whereas for the Schwaab procedure this appears to be.

When proving efficient, the proposed method that performs best can be (i) evaluated in the lab and can be (ii) used to evaluate if the diverging dynamics, momentarily observed for *Listeria* and *E. coli* K12, also exist for other microbial strains. As the dynamics described by the aCTMI are already observed for two different strains, similar observations might exist for other food-related strains.

So, under the assumption that the aCTMI is the correct one, two powerful different discrimination methods have been proven in simulation to select the correct model. This makes it relevant in future research to efficiently and accurately check whether aCTMI yields also in real life a better description of

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

the microbial behavior at low temperatures. And in this case to have optimal experimental design for robust and accurate estimation of the parameters and their variances of the aCTMI.

Acknowledgements

This research is supported by Projects OT/10/035, PFV/10/002 (Center-of-Excellence Optimization in Engineering) of the Research Council of the KU Leuven, and Project KP/09/005 (SCORES4CHEM) of the Industrial Research Council of the KU Leuven, and the projects FWO KAN2013 1.5.189.13 and FWO-G.0930.13 of the Research Foundation Flanders (FWO) and the Belgian Program on Interuniversity Poles of Attraction, initiated by the Belgian Federal Science Policy Office. J. Van Impe holds the chair Safety Engineering sponsored by the Belgian chemistry and life sciences federation essenscia. The scientific responsibility is assumed by its authors.

References

A. Atkinson, V. Fedorov, The design of experiments for discriminating between two rival models, Biometrica 62 (1975) 57–70.

D. Ucinski, B. Bogacka, T-optimum designs for discrimination between two multiresponse dynamic models, Journal of the Royal Statistical Society 67 (2005) 3–18.

M. Schwaab, J. Monteiro, J. Pinto, Sequential experimental design for model discrimination. taking into account the posterior covariance matrix of parameter uncertainties, Chemical Engineering Science 63 (2008) 2408–2419.

B. Donckels, D. De Pauw, B. De Baets, J. Maertens, P. Vanrolleghem, An anticipatory approach to optimal experimental design for model discrimination, Chemometrics and Intelligent Laboratory Systems 95 (2009) 53–63.

L. Rosso, J. R. Lobry, J. P. Flandrois, An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model, Journal of Theoretical Biology 162 (1993) 447–463.

Y. Le Marc, V. Huchet, C. Bourgeois, J. Guyonnet, P. Mafart, D. Thuault, Modelling the growth kinetics of listeria as a function of temperature, ph and organic acid concentration, International Journal of Food Microbiology 73 (2002) 219–237.

S. Bajard, L. Rosso, G. Fardel, J. Flandrois, The particular behaviour of listeria monocytogenes under sub-optimal conditions, Journal of Food Microbiology 29 (1996) 201–211.

E. Van Derlinden, J. F. Van Impe, Modeling growth rates as a function of temperature: Model performance evaluation with focus on the suboptimal temperature range, International Journal of Food Microbiology (2012).

D. Uciski, B. Bogacka, A functional experimental design factor for optimum discrimination between multiresponse models, in: Proceedings of the 5th Workshop on Simulation. St Petersburg, June 26 - July 2, 2005.

E. Walter, L. Pronzato, Identification of Parametric Models from Experimental Data, Springer, Masson, 1997.

E. Van Derlinden, K. Bernaerts, J. F. Van Impe, Accurate estimation of cardinal temperatures of Escherichia coli from optimal dynamic experiments, International Journal of Food Microbiology 128 (2008) 89–100.

D. Telen, F. Logist, E. Van Derlinden, I. Tack, J. Van Impe, Optimal experiment design for dynamic bioprocesses: a multi-objective approach, Chemical Engineering Science 78 (2012) 82–97.

L. Pronzato, E. Walter, Robust experiment design via maximin optimization, Mathematical Biosciences 89 (1988) 161 – 176.

J. A. Jacquez, P. Greif, Numerical parameter identifiability and estimability: Integrating identifiability, estimability, and optimal sampling design, Mathematical Biosciences 77 (1985) 201 – 227.

I.-C. Chou, E. O. Voit, Recent developments in parameter estimation and structure identification of biochemical and genomic systems, Mathematical Biosciences 219 (2009) 57 – 83.

B. Chen, S. Asprey, On the design of optimally informative dynamic experiments for model discrimination in multiresponse nonlinear situations, Industrial and Engineering Chemistry Research (2003) 1379–1390.

K. P. Burnham, D. R. Anderson, Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach, second ed., Springer, New-York, 2002.

Journal homepage: http://www.journals.elsevier.com/mathematical-biosciences/
Original file available at: http://dx.doi.org/10.1016/j.mbs.2014.01.006

C. Hurvich, C. Tsai, Regression and time series model selection in small samples, Biometrica 76 (1989) 297–307.

R.M. Kuiper, H. Hoijtink, and M.J. Silvapulle. An akaike-type information criterion for model selection under inequality constraints. *Biometrika*, 98:495–501, 2011.

Silvapulle, M.J., and Sen P.K. *Constrained statistical inference: Inequality, order and shape restrictions.* John Wiley & Sons Ltd, Hoboken NJ USA, 2005.

J. Baranyi, T. Roberts, Predictive microbiology - quantitative microbial ecology in food, Acta Alimentaria 34 (2004) 335–337.

J. Baranyi, T. A. Roberts, A dynamic approach to predicting bacterial growth in food, Internatinal Journal of Food Microbiology 23 (1994) 277–294.

E. Van Derlinden, K. Bernaerts, J. F. Van Impe, Simultaneous versus sequential optimal experiment design for the identification of multi-parameter microbial growth kinetics as a function of temperature, Journal of Theoretical Biology 264 (2010) 347–355.

T. Coleman, Y. Li, An interior, trust region approach for nonlinear minimization subject to bounds, SIAM Journal on Optimization 6 (1996) 418–445.

C. Audet, J. E. Dennis Jr, Analysis of generalized pattern searches, SIAM Journal on Optimization 13 (2003) 889–903.